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Mechanisms Linking Mechanotransduction and Cell Metabolism

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Abstract

Throughout their lifetimes, all cells experience force. These forces are sensed by cell surface adhesion receptors, such as the cadherins and integrins. Much attention has focused on identifying how these adhesion receptors transmit force. In contrast, less is known regarding how these force-activated pathways are integrated with other cellular processes. In this review, we describe how cadherins and integrins transmit force, and discuss how these adhesion receptors are linked to cell metabolism. We focus on understanding this connection by highlighting how the cadherins and integrins interact with a master regulator of energy homeostasis, AMP-activated protein kinase (AMPK) and its upstream activator, Liver Kinase B1 (LKB1). We consider why there is a need for force transmission to be coupled to metabolism and highlight the major unanswered questions in the field.

Introduction

Cells respond to numerous forces, such as shear stress, compression, stretching, as well as internally generated tension. These forces are sensed by cell surface adhesion receptors, such as cadherins and integrins, which are physically connected to the cytoskeleton through interactions with actin associated proteins. In response to force, both integrins and cadherins: (1) cluster, (2) recruit a similar repertoire of proteins, and initiate signaling cascades that culminate in activation of the small GTPase, RhoA. RhoA indirectly regulates myosin II activity, which in conjunction with actin filaments, allows cells to respond to mechanical stimuli (Figure 1). This response includes generation of internal contractile forces, reorganization of the actin cytoskeleton, and growth of the associated adhesion complex—a process known as cell stiffening [1, 2].

How the cadherin and integrin adhesion complexes stimulate cell stiffening has been the subject of intense scrutiny. Integrins are heterodimers of alpha and beta subunits that bind to the extracellular matrix on the outside of the cell. On the inside of the cell, the integrin cytoplasmic tails recruit various actin binding proteins, such as talin and vinculin (Figure 1). In response to mechanical force, the integrin tails undergo conformational changes. These changes promote talin binding which in turn stimulates the integrins to adopt an active conformation, associating with the extracellular matrix. Force also causes integrins to stimulate transduction cascades on the inside of the cell. Key among the force-activated

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upstream kinases. In mammals, the major upstream kinase phosphorylating AMPK is LKB1 [11]. Once active, AMPK stimulates energy generating processes (glucose uptake and fatty acid oxidation) and decreases energy consuming processes (protein and lipid synthesis) [12]. The ability to monitor the energy status and shift metabolism to maintain homeostasis in cells and organisms has allowed AMPK to emerge as a master regulator of mammalian metabolism.

Links between cell-cell adhesion and AMPK

Links between energy metabolism and cell adhesion have remained largely unexplored. A recent study revealed that treatment of epithelial monolayers with shear stress or application of force directly to E-cadherin stimulates AMPK activation and recruitment to the E-cadherin adhesion complex [13]. AMPK activation, and its localization to cell-cell junctions, requires LKB1 [13]. Furthermore, AMPK is a component of a signal transduction cascade culminating in contractility. In this signal transduction cascade, E-cadherin triggers Abl-mediated phosphorylation of Y822 vinculin leading to RhoA activation and phosphorylation and activation of myosin II (Figure 2). Inhibition of LKB1 or AMPK prevents force-induced Abl activation, vinculin phosphorylation, GTP-loading of RhoA, and myosin II phosphorylation. These observations validate AMPK as an upstream modulator of contractility at cell-cell contacts [13].

While we highlight the role of AMPK in E-cadherin mechanotransduction, other evidence links AMPK and LKB1 to E-cadherin. First, AMPK activators suppress the loss of E-cadherin and subsequent cell-cell adhesion that accompanies the transition of cancer cells from an epithelial to mesenchymal phenotype [14–18]. Second, AMPK is required to maintain two E-cadherin dependent processes in epithelial cells—(1) polarity and (2) barrier function [19–21]. In further support of role, AMPK is now generally appreciated to maintain cell-cell contacts in tissues, including the brain, heart, intestine, and kidney [22–26]. Third, the idea that AMPK lies downstream of E-cadherin is also supported by studies of the role of folliculin—a protein associated with the Birt-Hogg-Dube—a disease characterized by lung collapse and tumor formations in the kidney, colon and skin. Folliculin binds AMPK [27], and disease mutations in the folliculin gene produce truncated proteins that do not bind AMPK [28]. Interestingly, AMPK cannot be activated in epithelial cells in the absence of folliculin. The mechanism for the lack of AMPK activity is not well understood but is linked to changes in E-cadherin expression and localization to cell-cell adhesions. Also of note, deletion of folliculin in the lung epithelium leads to cellular apoptosis, alveolar enlargement, and impaired alveolar epithelial barrier function [29]. All of these events are linked to aberrant force transmission, suggesting the folliculin studies may warrant reconsideration in light of the newer studies. Taken together, the observations establish AMPK and LKB1 as bonafide E-cadherin effectors.

Why do cell adhesion molecules activate AMPK?

In response to force, E-cadherin activates AMPK culminating in the uptake of glucose and its oxidation to ATP. Many cellular processes require ATP, and older work suggests the actin cytoskeleton is one of them. Indeed, studies of resting platelets indicate approximately 50%

of the total ATP consumed in cells is needed to support actin cytoskeleton [30]. It could be argued that this estimate might be too high as platelets have a higher turnover of cytoplasmic ATP than most cells. However, similar studies of live neurons verified that 50% of the cytoplasmic ATP is needed to support the actin cytoskeleton [31]. The latter study went a step further to demonstrate the requirement for ATP is independent of the energy used by the $\text{Na}^+\text{-K}^+\text{-ATPase}$ —a major energy consumer in ionic homeostasis [31]. All of these measurements were made in cells in culture which are not expected to be dramatically rearranging their actin cytoskeletons. In contrast, force triggers robust actin cytoskeletal polymerization and rearrangements [32, 33]. Hence, cells under force require vast amounts of ATP to support the polymerization and rearrangement of actin and the amount required is likely to be higher than the estimates from platelets and neurons suggest.

Estimates for the amount of energy the actin cytoskeleton consumes to respond to force are not available. However, epithelial cells exposed to shear stress exhibit a 3.8-fold increase in actin deposition in cell-cell junctions [13]. This increase in F-actin enrichment requires AMPK. Inhibiting either AMPK or the AMPK-derived energy prevents cells from reinforcing their actin cytoskeletons [13]. Thus, epithelial cells under force activate AMPK to intensify their metabolism to provide the energy necessary to allow for F-actin reinforcement at cell-cell junctions.

It is tempting to speculate that the AMPK-derived ATP could also be used to support the sliding of myosin along actin filaments. In non-muscle, mammalian cells, myosin II is the major isoform. Upon phosphorylation at serine 19, myosin II generates force by binding to and sliding along actin filaments—a process that requires the hydrolysis of ATP. In muscle cells, vast amounts of ATP are needed to support contraction. However, myosin is unlikely to be a major energy drain in non-muscle cells as they contain very little myosin and far less of it is bound to actin filaments. Estimates of the molar ratio of actin to myosin in muscle cells are 6:1. In stark contrast, this molar ratio increases to 100:1 in non-muscle cells [34]. Hence, the sliding of non-muscle myosin II along actin filaments is not expected to represent a major energy drain in non-muscle cells.

It is also plausible that AMPK has effects independent of its ability to stimulate energy production. AMPK is a serine/threonine kinase that can phosphorylate many targets. Some of these targets are regulators of RhoA or myosin—two proteins critical for responding to force. For example, in a dividing cell, myosin II and actin accumulate midway between the poles of the spindle and align into a contractile ring which generates the constricting force to separate one cell into two cells. Both active AMPK and serine 19 phosphorylated myosin II localize to the mitotic spindle [35]. AMPK depletion reduces the amount of phosphorylated myosin associated with the spindle pole and decreases spindle alignment. In addition, there is some evidence that indicates AMPK directly phosphorylates myosin regulatory light chains [19]. However, this work has been called into question because the myosin light chain regulatory subunits do not have a consensus phosphorylation site for AMPK [36]. Furthermore, other studies reveal that the commercially available recombinant AMPK used to demonstrate direct phosphorylation of the myosin light chain regulatory subunits is contaminated with other kinases. In support of this notion, pure AMPK did not efficiently phosphorylate myosin light chains [36]. Alternatively, AMPK could control the

dephosphorylation of the myosin regulatory light chains. An AMPK-related kinase, known as NUA1, phosphorylates and inactivates the myosin phosphatase, suggesting that AMPK could perform a similar type of regulation [37]. Finally, it is equally plausible that AMPK affects myosin by modulating the function of its upstream activators. In support of this possibility, LKB1, an upstream AMPK activator, binds to the guanine nucleotide exchange factor, p114RhoGEF [38], and AMPK phosphorylates RhoA at Ser188, thereby reducing Rho-Rock signaling [39]. Taken together these observations indicate that AMPK directly or indirectly modulates the phosphorylation of myosin II.

Links between cell-matrix adhesions and AMPK

AMPK is also emerging as a modulator of integrin-mediated events. AMPK is a component of the integrin adhesome [40]. In addition, AMPK localizes to the leading edge of migrating cells—a locale where integrin function is well characterized [41]. Cells respond to migratory cues by extending a leading edge or protrusion in the direction of the migratory cue. These protrusions contain thin, sheet-like membrane protrusions known as lamellipodia. The lamellipodia are rich in a dense branched network of actin filaments. The leading edge protrudes by polymerizing new actin filaments and disassembling older filaments behind the leading edge. The growing actin filaments are thought to provide the force necessary to protrude and push the cell membrane forward [42]. In addition to actin networks, the leading edge also contains small nascent integrin adhesions. These adhesions turnover rapidly or give rise to focal complexes, which anchor the protrusion to the extracellular matrix behind the leading edge. In turn, the adhesions develop into more mature adhesions known as focal adhesions which assemble from near the front of the cell to its rear. In addition, some cells form fibrillar adhesions—stable and elongated adhesive structures that are not prominent in rapidly migrating cells [43].

Intense effort devoted towards understanding how cells migrate has revealed a role for AMPK. The exact nature of this role remains to be determined. Accumulating evidence indicates that AMPK modulates integrins directly and indirectly through effects on the actin cytoskeleton. The leading edge of migrating cells has increased levels of mitochondria and mitochondrial-derived ATP when compared to the cell body [41]. The increased ATP levels are accompanied by a significantly lower ATP:ADP ratio which triggers activation of AMPK. In this cellular region, active AMPK increases mitochondrial flux, ATP levels, and cytoskeletal dynamics; its inhibition suppresses cell migration and invasion. Another study describes a requirement for AMPK in integrin-mediated events. Indeed, AMPK is required for the reorganization of the actin cytoskeleton that supports monocyte adhesion to adhere to endothelial cells (Figure 3) [44]. Hence, AMPK positively modulates actin dynamics and protrusive events that occur in actively adhering and migrating cells.

Other studies indicate that AMPK inhibits integrins and cell migration. In support of an inhibitory role, AMPK was identified in an RNAi screen of proteins negatively regulating integrin activity [45]. Subsequent studies confirmed increases in integrin activation, fibrillar adhesion formation and mechanotransduction when AMPK is inhibited [45, 46]. AMPK also modulates actin and microtubule polymerization and decreases the rate and persistence of cell migration by phosphorylating the actin binding proteins VASP [47] and PDLim5, and the

microtubule binding protein CLIP-170 [48, 49]. Additionally, AMPK phosphorylates and targets the endosomal trafficking protein—sorting nexin 17 (SNX17)—for degradation, culminating in decreased $\beta 1$ and $\beta 5$ integrins at the plasma membrane [50, 51]. Taken together, these observations indicate that AMPK inhibits integrins and integrin-mediated events and are in contrast to the role of AMPK in modulating integrin function in the leading edge of migrating cells

There is a possible explanation for the discrepancy in the requirement for AMPK in integrin-mediated events. The evidence supporting a role for AMPK in cell migration examines AMPK in the leading edge of cells or in actively adhering cells—two settings that require energy intensive, actin polymerization and protrusive formation. In contrast, the data demonstrating AMPK plays an inhibitory role are largely confined to mature focal adhesions and fibrillar adhesions which are absent from the leading edge and are not expected to increase the energy burden. Taken together these findings suggest AMPK positively regulates integrin events and the actin cytoskeleton in the leading edge of cells and inhibits these processes in more distal regions of the cell where the more mature adhesive structures reside. Hence, it is possible that AMPK plays different roles depending on its subcellular localization and its mechanical environment. Different subcellular functions for other proteins have been described in migrating cells. Most well characterized among these is RhoA, which must be inhibited at the leading edge to allow for membrane protrusion and activated at the rear of the cell to promote migration. An alternative possibility is the different cell types contribute to the phenotypic differences. Numerous studies have shown that AMPK does not function similarly, or through identical targets, in different cells [52]. More work is needed to resolve these complexities.

Conclusions and Future Directions

Progress in the field of mechanotransduction has been substantial in the recent years. This work reveals a surprising connection between the adhesive machinery and the enzymes that regulate mammalian metabolism. These new findings precipitated a closer inspection of older work and have revealed that other connections between cell adhesion and cell metabolism exist. Furthermore, it is increasingly apparent that a cell's response to force requires energy—with significant levels of energy being used to reinforce the actin cytoskeleton.

With these new findings, exciting questions linking cellular mechanics and metabolism are open for discussion. Key among these questions is whether other cellular processes that involve acute actin polymerization activate AMPK to provide energy to sustain these events. Additionally, it remains unclear how many linkages there are between the adhesive and metabolic machineries. The integrin adhesome contains 43 gene products with metabolic function [40], and the cadherin adhesome contains 52 metabolic proteins [53]. Hence, it is likely that AMPK is only a small perceptible component of pathways that remain to be uncovered. Finally, metabolism is experiencing a renaissance because disturbances in metabolic regulation are increasingly appreciated as a cause of disease. Hence, it will be important to investigate the linkages between cell metabolism and mechanotransduction in

diseases such as cancer, diabetes and obesity which are accompanied by losses in cell adhesion and metabolic reprogramming.

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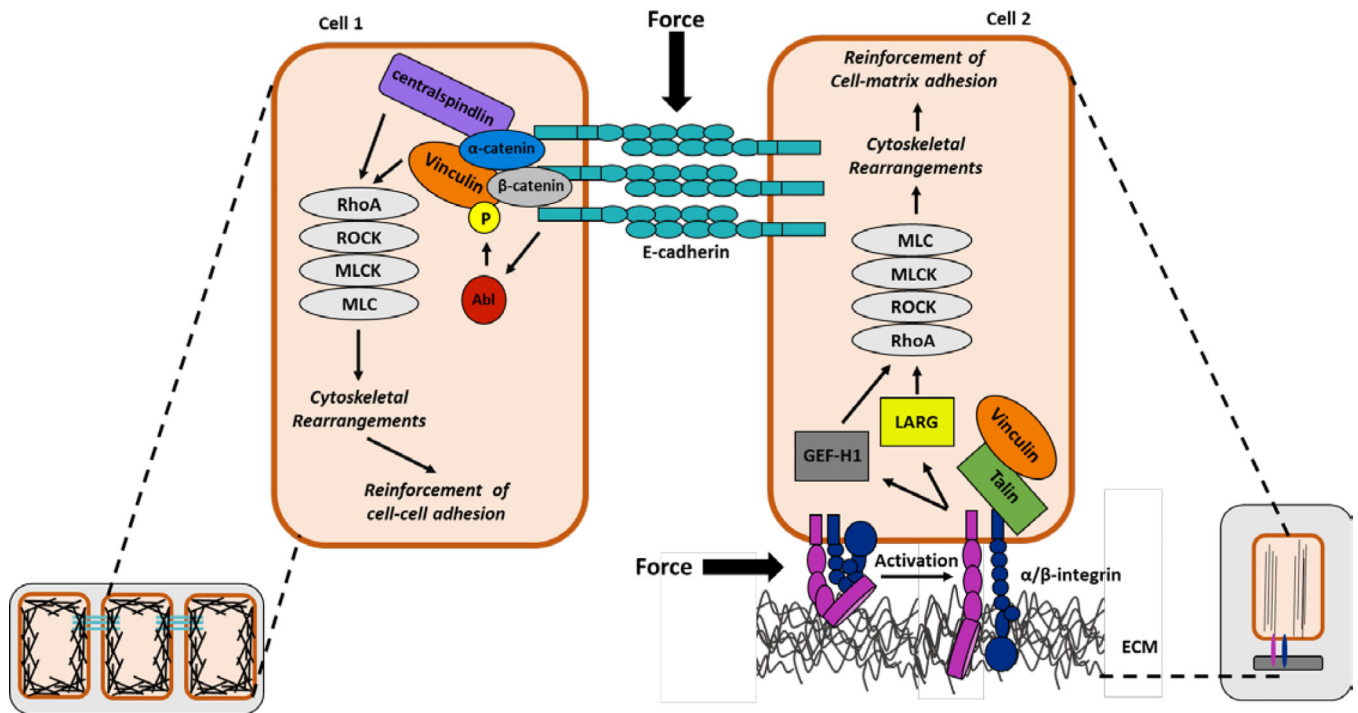


Figure 1: Integrin- and cadherin-mediated mechanotransduction pathways.

At sites of cell-cell contact, the extracellular domains of E-cadherin bind to E-cadherins on neighboring cells to provide strong cell-cell adhesion, while the cytoplasmic domain recruits catenins, which in turn associate with additional cytoskeletal and regulatory proteins, such as vinculin and centralspindlin. In response to force, cadherin induces the activation of Abelson tyrosine kinase (Abl) which leads to the phosphorylation of vinculin at Y822. This signaling event is necessary for the activation of the Rho GTPase pathway, ultimately leading to cadherin-mediated cell stiffening. At cell-matrix adhesions activated integrins interact with the extracellular matrix on the outside of the cell, which triggers activation of intracellular signaling and recruitment of actin binding proteins, such as talin and vinculin. In response to force, the integrins recruit and activate two distinct signaling pathways that trigger recruitment of two RhoA guanine nucleotide exchange factors, LARG and GEF-H1. These integrin-mediated signaling events are critical for the force-induced activation of RhoA and the reinforcement of integrin-actin linkages at the cell-matrix adhesions. Abl=Abelson tyrosine kinase; ROCK=Rho associated protein kinase; MLCK=myosin light chain kinase; MLC=myosin light chain; GEF-H1=guanine nucleotide exchange factor H1; LARG=leukemia-associated Rho-GEF; ECM=extracellular matrix

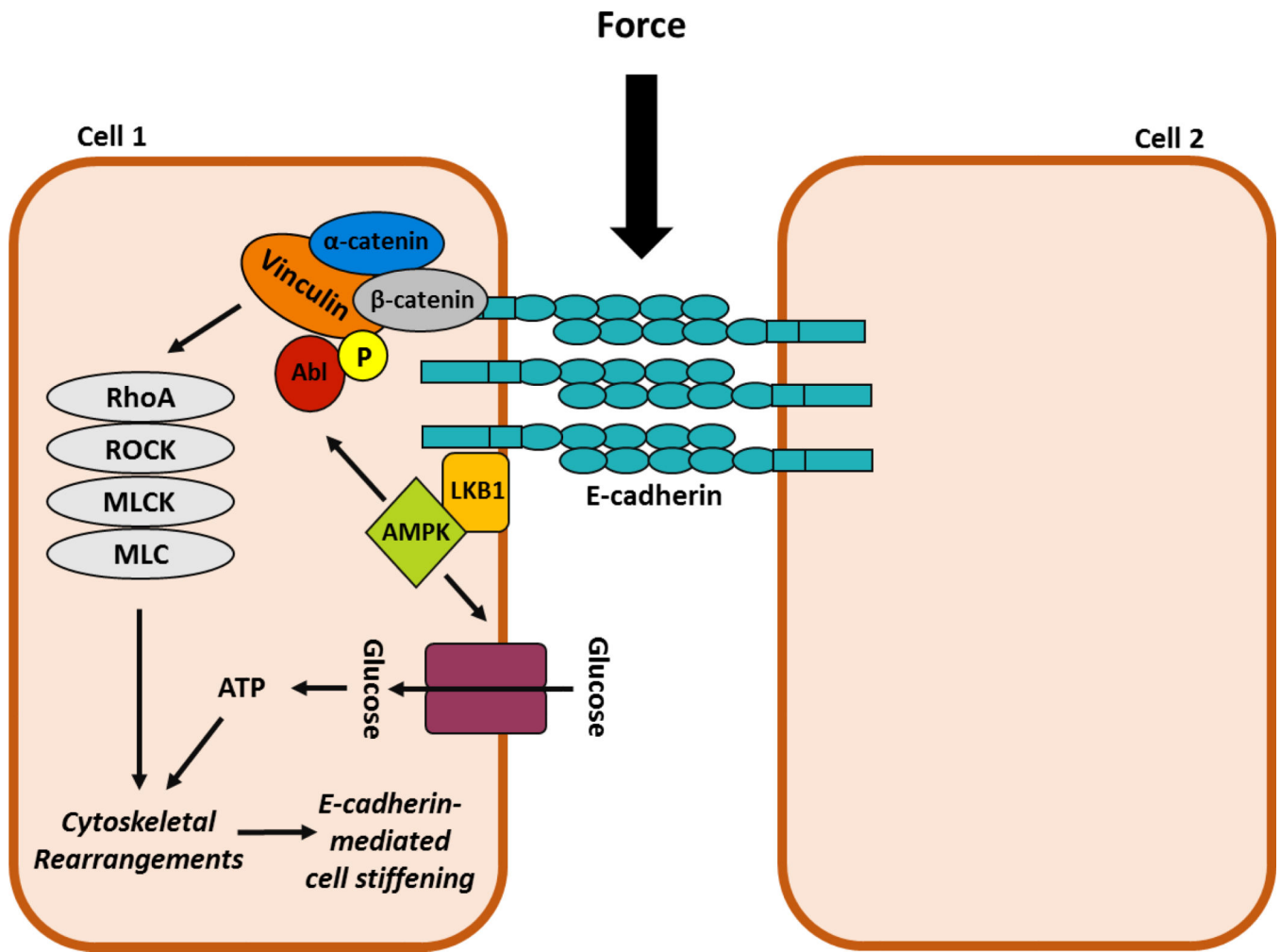


Figure 2: E-cadherin-mediated force transmission stimulates AMPK culminating in energy production.

Force on E-cadherin triggers the recruitment LKB1 and the subsequent activation of AMPK. Activated AMPK has two effects. First, it stimulates a signal transduction cascade that includes Abl-mediated phosphorylation of Y822 vinculin (yellow circle) and the subsequent activation of a RhoA-ROCK-MLCK pathway that leads to increased MLC phosphorylation and elevated contractility. Second, AMPK stimulates glucose uptake and oxidation to ATP to provide energy to allow the actin cytoskeleton to reorganized. Both signaling events are necessary for cell stiffening. LKB1=liver kinase B1; AMPK=AMP- activated protein kinase; Abl=Abelson tyrosine kinase; ROCK=Rho associated protein kinase; MLCK=myosin light chain kinase; MLC=myosin light chain.

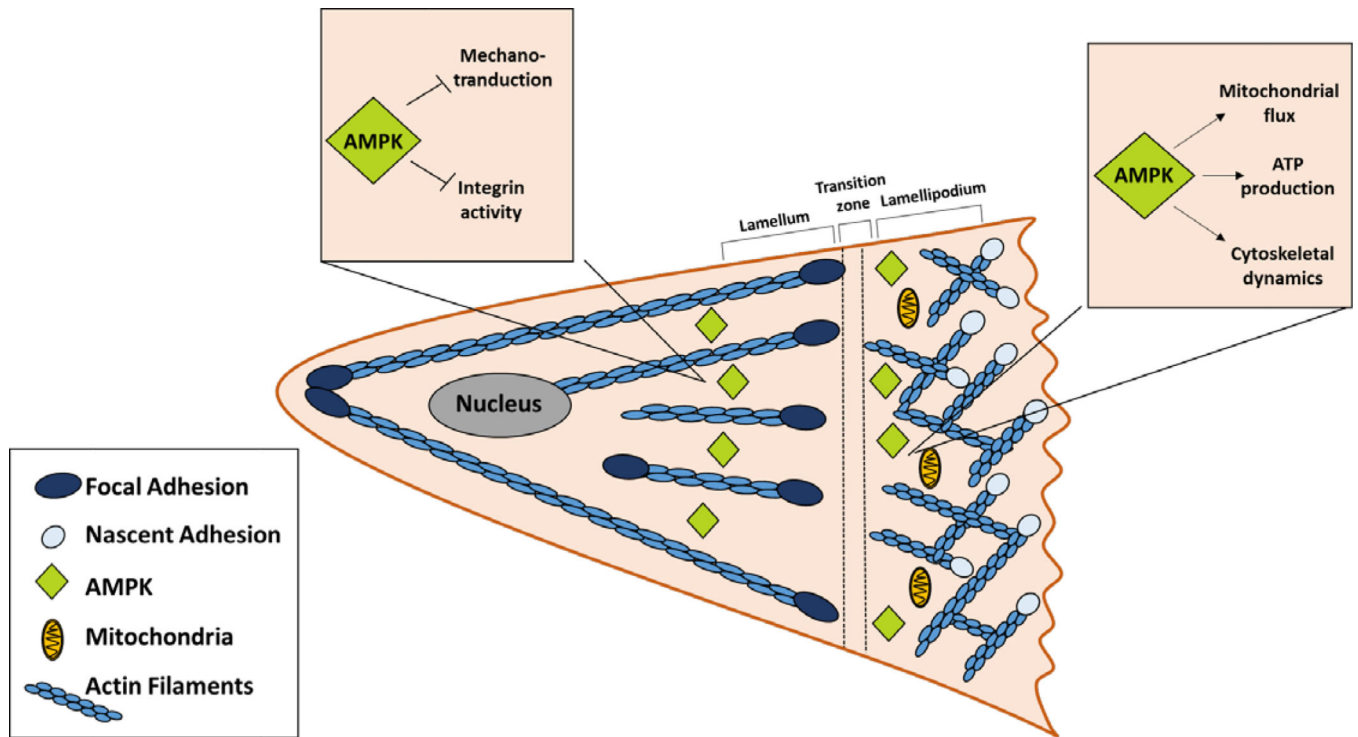


Figure 3: Model for the actions of AMPK in a migrating cell.

The leading edge or lamellipodium of a migrating cell is integrin-rich nascent adhesions and mitochondria. In this region, AMPK stimulates mitochondria flux, ATP production and cytoskeletal dynamics. Behind the leading edge is a transition zone where the nascent adhesions mature or disassemble. Mature focal adhesions are present further back in the cell in the lamellum. In mature adhesions, AMPK inhibits integrin activity and mechanotransduction.